Samples

CRP-containing samples of varying CRP concentration were prepared from a 200 mg/l of recombinant CRP (Fitzgerald) in hCRP depleted serum.

Procedure

15 µl of CRP-containing serum diluted 1/20 in test buffer (50 mM borate buffer pH 8.0, 3% BSA, 5% sucrose, 0.15 M NaCl, 0.005% $CaCl_2$, 0.05% NaN_3) were applied to the application zone of the membrane strip. Then, 15 µl of detection conjugate solution [anti-CRP monoclonal antibody (Fitzgerald) coupled to 0.1 µm TransFluoSpheres-SO_4/CHO (633/720 nm) (Molecular Probes Inc.), the above test buffer] were added, the amount of anti-CRP conjugate being 3 µg per test strip which was a 15 x molar excess in relation to the highest standard value. The conjugate addition was followed by a wash with 15 µl of test buffer. The fluorescence of the strip was then measured. The results are shown in Table 2 below.

Table 2

CRP conc. (mg/l)	Peak area obtained (V x mm)
0	0.41
0	0.60
10	7.51
10	7.130
20	8.86
20	9.42
40	11.97
40	10.67
80	11.70
80	12.91
200	14.27
200	14.16

The above Examples 1 and 2 thus demonstrate that it is possible to run an assay on undiluted high concentration samples without using huge amounts of reagents when using the methodology of the present invention.